PROTEIN HYDROLYSATE EFFECT ON ALIMENTARY HYPERCHOLESTEROLEMIA AND LIPOIDOSIS OF THE AORTA IN RABBITS

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Changes in protein metabolism along with lipid exchange disorders have an essential practical bearing on the development of atherosclerosis. It is a matter of dysproteinemia in the plasma proteins — a reduction of albumins and substantial rise of globulins (2, 3, 7, 8, 15), decreased protein synthesis in the arterial wall (5), reduced content of soluble protein in the liver and aorta (3), and diminished methionine accumulation in the organs of atherosclerotic animals (5, 10).

On the other hand, an ever increasing number of authors underscore the effect of proteins and amino acids on serum cholesterol level and lipid infiltration in the organs (4, 6, 9, 17, 18, 20). S.-j. C. Yeh et al (1973) found a quicker turnover of the plasma cholesterol, and enhanced fecal excretion of neutral steroids and cholic acids in rich in proteins diets. Y. Itokawa et al (1973) observed a marked hypocholesterolemic effect of sulfur-containing amino acids in experimental atherosclerosis in rats. According to J. Kenney et al (1973), chickens fed a poor protein diet have a higher cholesterol level in the plasma and liver as compared to those fed a diet rich in proteins. I. Popdimitrov and K. Demireva (1975) found an elevated methionine — ^{75}Se accumulation in animals where cholesterol nutrition was supplemented by protein hydrolysate.

Having in mind the literature data outlined above, we set out to verify the influence of protein hydrolysate "Hydroprot" (11) on serum lipids, and on the development of lipoidosis in the aorta of rats with experimentally induced atherosclerosis.

Material and Methods

The study was performed in a series of 243 male rabbits with weight ranging from 2000—2700 g, divided into three groups: I — with experimentally induced atherosclerosis after the method of Anichkov (each animal received 0.2 g/kg weight cholesterol, dissolved in sunflower — seed oil added to the food; II — with experimental atherosclerosis and daily subcutaneous injection of protein hydrolysate at dose 5 ml/kg weight, and III — controls. Groups I and III were injected with physiological saline at dose analogical to that in protein hydrolysate. Prior to the experiment, and periodically at 15-day intervals, venous blood was taken for serum lipids' investigation. The animals were killed at 90 days after the beginning of the experiment through exsanguination. Atherosclerotic changes along the intima of the aorta were recorded planimetrically after the method of G. G. Avtandilov (1963) in part of the laboratory animals.

Serum cholesterol determination was carried out according to the method of S. Ilca (1962), beta-lipoproteins — after Burstein and Samaille, total lipids — after the method of Bloor as modified by J. H. Bragdon (1951), and phospholipids — after the method of A. Svanborg and Z. Svennerholm (1961).

Results

Changes in the serum cholesterol level, followed up in dynamics, are presented in Fig. 1. The animals of group I display a rapid and considerable increase in cholesterol. The difference relative to controls proves to be statisti-

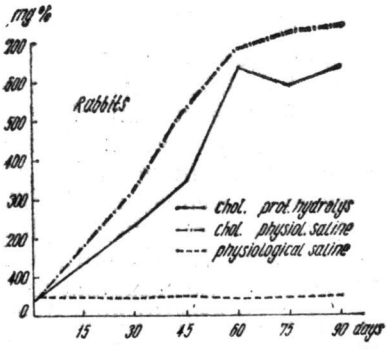

Fig. 1. Changes in serum cholesterol level in rabbits with experimental atherosclerosis under the influence of protein hydrolysate

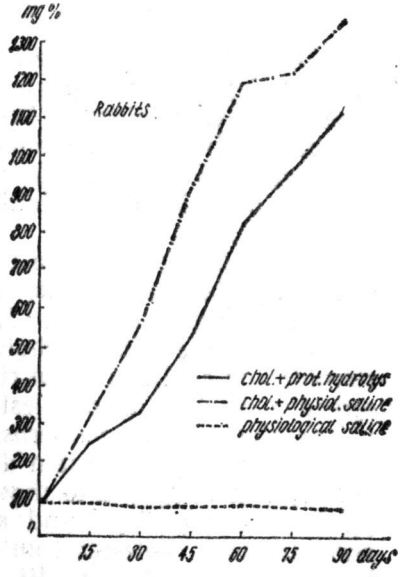

Fig. 2. Protein hydrolysate effect on the level of beta-lipoproteins in the serum of rabbits with experimental atherosclerosis

cally reliable ($p < 0.01$) within 15 days. The cholesterol rise is steady and uninterrupted till the end of the experiment (from 37.8 mg % it grows to 745.5 mg %) steeper in the beginning, and less pronounced after the 60th day. In the animals of group II, injected with protein hydrolysate, cholesterol is maintained at a lower level throughout the entire observation period. During the first 15 days and at 60 days the differences are less marked, while the greatest difference is recorded at 45 ($P < 0.001$) and 60 days ($P < 0.001$).

Beta-lipoproteins (Fig. 2) in group I show a steep rise till the end of the experiment, with a certain delay being recorded between the 60th and 75th day. In the protein hydrolysate group (II), following a 15-day adaptation period, a substantially weaker, wavy rise of beta-lipoproteins is observed, and within 90 days all the values are statistically significantly lowered as compared to the animals of group one.

The steepest increase is noted in the total lipids' level. Among the animals in group I the total increase lipids from 336.7 mg% to 2238.8 mg%, while in group II the increase observed is smaller (up to 1895.7). A reliable difference compared to group I is established in all periods of study, except for the first fifteen days (Fig. 3).

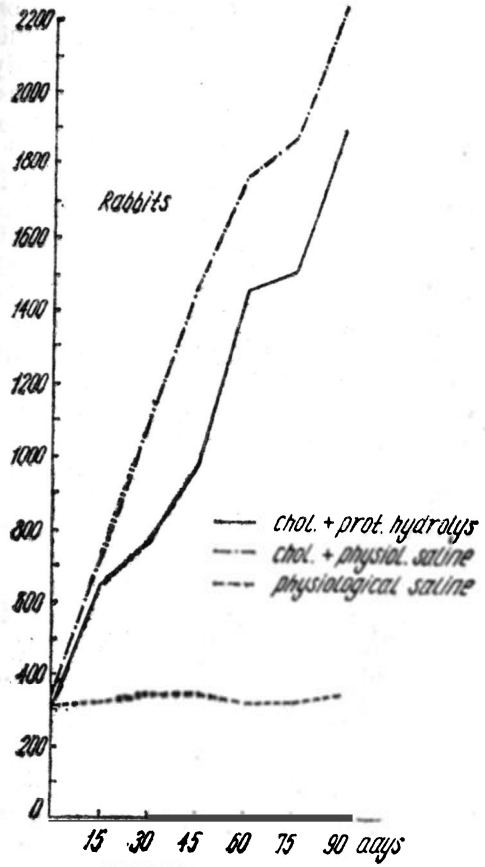

Fig. 3. Changes in total lipids' level in the serum of rabbits with experimentally induced atherosclerosis, receiving protein hydrolysate

In phospholipids alone the increase in group I is less pronounced as compared to the animals of group two. A certain decrease occurs about the 75th day, followed by an increase. The data concerning group II show a uniform and steady rise parallel to the lengthening of the treatment period (Fig. 4).

The morphological changes in the aorta of the animals of group I, measured with a planimetric ruler, consist in atherosclerotic alterations ranging

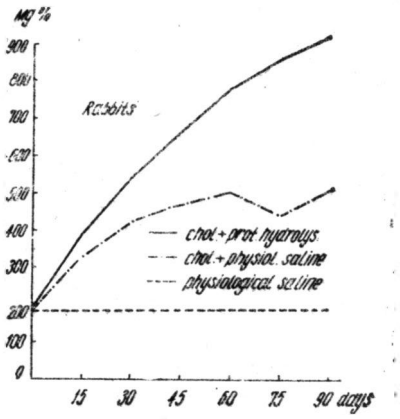

Fig. 4. Changes in the level of serum phospholipids in rabbits with experimental atherosclerosis under the effect of protein hydrolysate

from 32.3 per cent to 56 per cent, and in isolated cases — up to 80 per cent of the whole surface of the intima. Entire fields of confluent, rather clearcut, rough atherosclerotic changes are observed. The lipid deposits are most strongly pronounced in the arcus and chest tract of the aorta, they are slightly less manifested in the ascending part, and least to virtually absent — in the abdominal aorta. The animals receiving protein hydrolysate (group II) show substantially less pronounced to absent lipid deposits. The changes fluctuate from 0.5 per cent to 15.2 per cent, and in sporadic cases they involve up to 35 per cent of the intima surface. They are located mainly around the openings of the carotid artery and thoracic aorta. The lipid deposits are not roughly outlined, and reveal the form of single droplets or minute fields.

Discussion

Our results show that protein hydrolysate administered to rabbits fed cholesterol diet exerts up to a great extent a protective action against atherosclerotic changes in the vessels. The fact that protein hydrolysate introduction leads to maintaining the serum lipids at a lower level, as compared to the animals of group I, is of utmost importance. On the other hand, the constantly increasing phospholipids in their capacity of stabilizers of the colloid solutions, interfere with cholesterol deposition. Presumably the latter effect might be ascribed to the protective influence exerted by protein hydrolysate amino acids on the functions of the liver and other organs related to lipid metabolism which is also supported by the histomorphological changes found by E. Maleva and K. Demireva (1975). The presence of a complete constellation of irreplaceable and replaceable amino acids in the protein hydrolysate "Hydroprot" in all likelihood exerts a hydrocholesterolemic effect by way of the sulfur-containing amino acids' effect, established by Y. Itokawa et al (1973), or by means of activating the cholesterol turnover, established by S-j. C. Yeh et al (1973). Moreover, the stronger methionine ^{75}Se accumulation, demonstrated by I. Popdimitrov and K. Demireva (1975) in the organs of protein hydrolysate-treated animals under analogical experimental conditions, is interpreted as an indirect proof for a favourable influence on protein metabolism also. According to M. G. Kritzman (1967) the changes in protein metabolism appear to be the contributing factor in a great number of diseases since the potential ability of a mutual transition of inactive into active protein is inherent of each protein structure thanks to the peculiar function of amino acids to form plastic material and biologically active substances. On the basis of the data referred to above the inference is reached that protein hydrolysate, applied to experimental atherosclerosis, exerts a protective action on protein and fat metabolism. Most probably, through the stabilization of protein structures within the organism, and first and foremost, enzyme systems (M. Shtereva and K. Demireva — 1973) it obviates the disorders in the metabolism of substances, lipids in particular. The results submitted in the work corroborate the opinion of a great number of authors about the pathogenic role played by protein metabolism disorders in the atherosclerotic process.

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ВЛИЯНИЕ БЕЛКОВОГО ГИДРОЛИЗАТА НА АЛИМЕНТАРНУЮ ГИПЕРХОЛЕСТЕРОЛЕМИЮ И ЛИПОИДОЗ АОРТЫ У КРОЛИКОВ

К. Демирева

РЕЗЮМЕ

Прослежено влияние белкового гидролизата „гидропрот" на уровень холестерола сыворотки, бета-липопротеидов, общих липидов и фосфолипидов у кроликов с экспериментальным атеросклерозом. Исследования проведены до опыта, на 15, 30, 45, 60, 75 и 90 день питания холестеролом и назначения белкового гидролизата.

Установлено, что после некоторого приспособительного периода к новому режиму питания, парентеральное применение белкового гидролизата у животных с экспериментальным атеросклерозом приводит к задерживанию уровня холестерола сыворотки, общих липидов и бета-липопротеидов на более низком уровне, чем у животных не получающих белковый гидролизат. Уровень фосфолипидов значительно выше до конца эксперимента. Атеросклеротические изменения интимы аорты выражены значительно слабее. Можно допустить, что этот результат обусловлен протективным воздействием аминокислот белкового гидролизата на нормальные функции печени и других органов, имеющих отношении к липоидному обмену.